CHEMICAL SCALE INVESTIGATIONS OF THE GATING MECHANISM OF ION CHANNELS

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In memory of

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ABSTRACT

The studies contained in this dissertation are aimed at utilizing chemistry to understand neurobiology and neuronal communication. Chapters 2 and 3 both address the gating of ion channels, describing structure-function studies to shed light on the gating mechanisms of two classes of ion channels. Chapter 2 studies the gating mechanism of the mechanosensitive channel of small conductance (MscS), which is voltage modulated. Elucidating the mechanism of voltage sensation in MscS may provide insight into how voltage-gated channels translate a change in membrane potential to channel gating. The research discussed in Chapter 2 is aimed at elucidating the role of two arginine residues, in the TM1 and TM2 of MscS, in voltage sensing. We generated two MscS mutants, Arg46Ala and Arg74Ala, to evaluate the effects of "neutralizing" the charged side chain on the voltage sensing ability of the channel. The mutants were evaluated using single channel analysis in *E. coli* spheroplasts. Our preliminary results indicated a potentially significant role for Arg46 in the voltage sensitivity of MscS, however this data set is not extensive due to inconsistency in the spheroplasts preparation.

In Chapter 3, we utilized nonsense suppression to incorporate unnatural amino acids to study the gating of the cation-selective Cys-loop family of ion channel receptors. Specifically, it describes work aimed at elucidating the role of *cis-trans* isomerization of a conserved proline residue in the gating mechanism of the serotonin-gated 5-hydroxy-tryptamine receptor 3A (5-HT_{3A}R) and the nicotinic acetylcholine receptor (nAChR). A series of proline analogues, of varying *cis* preference were incorporated at proline 308 in the M2-M3 loop of the 5-HT_{3A} receptor using *in vivo* nonsense suppression methodology in a *Xenopus* oocyte expression system. Electrophysiological analysis of the mutant channels revealed a linear relationship

between the *cis* preference of the proline analog and the EC_{50} of the mutant channel—suggesting that proline 308 may serve as a hinge during the gating of the 5-HT_{3A} receptor. From these data, we proposed a model of gating for the 5-HT_{3A} receptor. Initial results from similar studies in nAChR suggest that the analogous proline does not play a role in its gating. These results suggest that while the 5-HT_{3A} receptor and the nACh receptor are highly homologous and are members of the same superfamily of channels, they have evolved to utilize different mechanisms of gating.

Lastly, Chapter 4 addresses the role of fucose-galactose carbohydrates in learning and memory. It aims to identify lectins to fucose-α(1-2)-galactose as well as identify the corresponding glycoproteins bearing fucose-α(1-2)-galactose. Chemical probes were synthesized and used to study fucose-α(1-2)-galactose binding proteins. One of the probes was used to demonstrate the existence of fucose-α(1-2)-galactose binding proteins in hippocampal neurons. Furthermore, initial results from experiments with a photoreactive probe suggested that the design of our probe is sufficient to isolate fucose-α(1-2)-galactose binding proteins from the brain. Additionally, we were able to use antibodies specific to fucose-α(1-2)-galactose epitopes to examine fucose-α(1-2)-galactose bearing glycoproteins in the brain. Overall, results from both studies utilizing chemical probes and molecular probes strongly suggest that the modifications of proteins with fucose-α(1-2)-galactose epitopes and the expression of fucose-α(1-2)-galactose binding proteins are developmentally regulated.

TABLE OF CONTENTS

Acknowledgements iv

Abstract ix

Table of Contents xi

List of Figures and Tables xiii

Chapter 1: Introduction 1

1.1 Introduction 2

1.2 Chemical methodologies aimed at understanding protein function 3

1.3 Dissertation work 14

1.4 References 16

Chapter 2: Putative Voltage Sensing Arginines in the Mechanosensitive Channel of Small Conductance (MscS) 20

2.1 Introduction 21

2.2 Experimental design 34

2.3 Results 34

2.4 Discussion 42

2.5 Conclusions 44

2.6 Experimental methods and materials 44

2.7 References 49

Chapter 3: *Cis-Trans* Isomerization at a Conserved Proline during Gating of a Cys-loop Receptor.....55

3.1 Introduction.....56

3.2 Experimental design.....66

3.3 Results.....67

3.4 Discussion.....77

3.5 Conclusions.....80

3.6 Experimental methods and materials.....82

3.7 References.....121

Chapter 4: Fucose-galactose in Learning and Memory.....127

4.1 Introduction.....128

4.2 Experimental design.....133

4.3 Results.....137

4.4 Discussion.....152

4.5 Conclusions.....156

4.6 Experimental methods and materials.....158

4.7 References.....182

FIGURES AND TABLES

Chapter 1

Figure 1.1. Synaptic transmission between neurons 2

Figure 1.2. Nonsense suppression methodology 5

Figure 1.3. Nonsense suppression methodology in Xenopus oocytes.....7

Figure 1.4. Chemical acylation of tRNA to append unnatural amino acids.....8

Figure 1.5. Structures of unnatural amino acids that have been incorporated.....9

Figure 1.6. Three main classes of photoreactive agents 11

Figure 1.7. Two methods of generating photoreactive DNA 12

Figure 1.8. Photoreactive amino acids.....13

Chapter 2

Figure 2.1. Crystal structure of MscS.....25

Figure 2.2. Putative voltage sensing arginines in MscS 27

Figure 2.3. Schematic of a K_v channel subunit.....30

Figure 2.4. Three models of voltage sensing in voltage-gated ion channels.....33

Figure 2.5. Generation of giant spheroplasts from E. coli.....35

Figure 2.6. Schematic of voltage step protocol 37

Figure 2.7. ln(NP_{open}) vs. membrane potential for wild-type MscS 38

Figure 2.8. Diminished wildtype channel activity with each voltage step cycle 39

Figure 2.9. Activity comparison of wild-type MscS and R46A mutant MscS 40

Figure 2.10. Western blot analysis of wild-type and mutant MscS.....42

Chapter 3

Figure 3.1. Schematic of Cys-loop receptors.....58

Figure 3.2. Pin and socket mechanism of gating.....61

Figure 3.3. M2-M3 loop sequence alignment.....63

Figure 3.4. Cis-trans isomerization of proline.....64

Figure 3.5. Structures of incorporated proline analogues.....66

Figure 3.6. Synthesis of cis-5-tert-butyl-L-proline.....69

Figure 3.7. Traces for mutant 5-HT_{3A} receptors expressing Tbp.....71

Table 3.1. Data summary.....71

Figure 3.8. Linear free energy correlation.....72

Figure 3.9. Dose response curves for wild-type receptor at varying concentrations of MDL72222.....73

Figure 3.10. Schild plots.....74

Figure 3.11. pIC_{50} vs. –log (EC_{50}).....76

Figure 3.12. Proposed model of gating mechanism.....78

Table 3.2. Crystal data and structure refinement for LWL101 (CCDC 258350).....90

Table 3.3. Atomic coordinates.....97

Table 3.4. Bond lengths and angles.....99

Table 3.5. Anisotropic displacement parameters.....103

Table 3.6. Hydrogen coordinates.....105

Chapter 4

Figure 4.1. 2-Deoxy-D-galactose inhibits fucosylation 129

Figure 4.2. Mechanisms for fucosyl saccharides in learning and memory 132

*Figure 4.3. Structures of chemical probes **10** and **11*** 133

Figure 4.4. Schematic for using photoreactive probes 136

*Figure 4.5. Synthesis of probe **10*** 138

Figure 4.6. Confocal microscopic images 140

*Figure 4.7. Synthesis of probe **11*** 141

*Figure 4.8. Synthesis of PEG linker **18*** 142

*Figure 4.9. Data of probe **11** in embryonic hippocampal neurons* 144

*Figure 4.10. Data of probe **11** in adult hippocampal neurons* 145

Figure 4.11. Western blot analysis of cross-linked proteins 148

Figure 4.12. Synthesis of O-ethyl fucose galatose 150

Figure 4.13. Western blot analysis of competition experiments 151

Figure 4.14. Structure of the blood group antigen H type 2 152

Figure 4.15. Western blot analysis using A46-B/B-10 antibody 153